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Ultrastructural characterization of *Hexisopus psammophilus* (Arachnida: Solifugae: Hexisopodidae) spermatozoa in comparison to other solifuge spermatozoal traits

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Abstract. The family Hexisopodidae is endemic to southern Africa. Hexisopodids represent a very peculiar group of Solifugae. They differ from all other solifuge families through various autapomorpic adaptations to a subterranean mode of life, most notably the presence of fossorial legs. The phylogeny of the Solifugae is widely unresolved. The ultrastructure of spermatozoa has successfully been used for phylogenetic analyses in other animal taxa. Therefore, the question arose whether the morphological peculiarity of the family Hexisopodidae might also be reflected in the ultrastructure of their spermatozoa. This was investigated for *Hexisopus psammophilus* Wharton 1981 (Hexisopodidae). Spermatozoa do not seem to aggregate in the testes, nor in the vasa deferentia. Sperm cells are aflagellate, roundish, and with irregularly shaped chromatin bodies. Each sperm is surrounded by a secretion sheath, thus representing a typical cleistosperm, the first record of this form of sperm transfer in solifuges. The sperm cells form finger-like protuberances and contain putative granules of glycogen, features shared with the Ammotrechidae, Daesiidae and Solpugidae. The acrosomal complex shows additional similarity with the Solpugidae. Overall, the spermatozoa of *H. psammophilus* share some morphological features with the Ammotrechidae and Daesiidae, but mostly resemble that of the family Solpugidae.

Keywords: Camel-spider, sperm cell, fine structure, cleistospermia, phylogeny

Solifuges, also called camel-spiders, occur worldwide on all continents with the exception of Australia and Antarctica (Punzo 1998). They represent one of the mesodiverse arachnid orders comprising 12 families and about 1087 species (Harvey 2002, 2003). Also known for their great sprint speeds and voracious feeding behavior, knowledge about, for example, their systematics, phylogeny, behavior and life cycle is still superficial and fragmentary. Among the 12 families known to date, one family, Hexisopodidae (mole- or teddybear solifuges) strikingly differs morphologically from all other solifuge families through the presence of various autapomorphic characters, most notably their fossorial, rather than cursorial, legs. Highly modified adaptations such as these often obscure real relationships with other taxa, and the putative hexisopodid relationships are no exception. The family Hexisopodidae Pocock 1897 only occurs in southern Africa (mainly in South Africa and Namibia, but also in Angola, Zimbabwe, Zambia, and Botswana). Twenty-three described species are distributed among the two genera *Hexisopus* Karsch 1879 and *Chelypus* Purcell 1902 (Harvey 2003). The main morphological difference between these two genera is the presence of well developed spines on the pedipalps of *Chelypus*, whereas such structures are lacking in *Hexisopus* (Roewer 1934). Due to their primarily subterranean mode of life, the species of Hexisopodidae are extremely difficult to study in any aspect.

The current classification of solifuges does not recognize any subordinal or superfamilial arrangement (Harvey 2003). This classification, based mainly on the taxon delineation established by Roewer (1934), relies on highly variable characters not only at the genus level but at the species level as well. For these reasons, various authors have critically challenged the current Roewerian system (e.g., Muma 1951, 1976; Harvey 2002).

The ultrastructure of spermatozoa has been shown to contribute valuable characters to elucidate and to substantiate phylogenetic hypotheses in many animal groups (e.g., Alberti 1984, 1991; Baccetti 1970; Baccetti & Dallai 1978; Dallai & Afzelius 1995; Jamieson et al. 1999; Dallai et al. 2003; Downing Meisner et al. 2005; Liana & Litvaitis 2007). Within arachnids three different types of sperm cells are known: 1) filiform flagellate - present only in scorpions (da Cruz-Landim & Ferreira 1972, 1973; Alberti 1983; Vignoli et al. 2008; Michalik & Mercati 2010), 2) coiled flagellate - present in Amblypygi, Uropygi, Araneae, Pseudoscorpiones and Ricinulei (Alberti 1985, 1990, 2000; Alberti & Palacios-Vargas 1984; Callaini & Dallai 1993; Michalik et al. 2004a, 2004b; Michalik & Huber 2006; Talarico et al. 2008; Talarico & Michalik 2010) and 3) aflagellate - present in Acari, Solifugae, Opiliones and Palpigradi (Alberti 1980a, b, c, 2000, 2005a; Alberti et al. 2009; Klann et al. 2005, 2009).

The morphological variety of spermatozoa within these groups differs strongly. Spiders and mites exhibit a large diversity in, e.g., morphology and size of their spermatozoa (Alberti 1980a, b, 1990, 2000), whereas other groups such as scorpions, ricinuleids, and solifuges apparently show only slight variability in their characteristic ground patterns (Alberti 1983; da Cruz-Landim & Ferreira 1972, 1973; Talarico et al. 2008; Klann et al. 2009; Michalik & Mercati 2010). Nevertheless, within arachnids these results are only

included in modern phylogenetic analyses to a limited degree (Weygoldt & Paulus 1979a, b; Shultz 2007; Dabert et al. 2010; Pepato et al. 2010). Because many traditional morphological characters of solifuges have been shown to fail substantial systematic and phylogenetic requirements as already mentioned above, the ultrastructure of solifuge spermatozoa might be an important contribution towards proposing phylogenetic hypotheses, not only within solifuges, but also for higher level arachnid phylogeny. The present study aims to characterize the fine structure of *Hexisopus psammophilus* Wharton 1981 (Hexisopodidae) spermatozoa in order to reveal whether there are any spermatozoal traits that distinguish Hexisopodidae from other solifuge sperm cells.

METHODS

A male of *H. psammophilus* was collected at the Gobabeb Research Station, Namibia (23°34′40.5″S, 16°02′31.3″E) (Voucher specimen deposited at the National Museum of Namibia, SMN 14218). The male genital system was removed and preserved in 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.2, 1.8% sucrose) at 4° C overnight. Thereafter, the specimen was rinsed in phosphate buffer and post-fixed in 2% aqueous OsO_4 at 4° C for 2 h. After being rinsed in buffer, the male genital system was dehydrated in graded ethanol series (60%-absolute). The specimen was embedded in Spurr's medium (Spurr 1969). Semi-thin sections were made for general orientation and stained according to Richardson et al. (1960). Ultrathin sections (50–70 nm) were made using a Diatome diamond knife. These sections were placed on uncoated copper grids and stained with saturated uranyl-acetate in 70% methanol and lead-citrate according to the method after Reynolds (1963). Transmission electron microscopy was performed with a JEOL-JEM-1011.

Abbreviations: AF = acrosomal filament, AV = acrosomal vacuole, bL = basal lamina, C = centriole, CB = chromatin body, Epi = epithelium, Gly = glycogen, Hd = hemidesmosome, Lu = lumen, Mi = mitochondrium, MlB = multilamellar body, Mu = muscle, Mv = microvilli, N = nucleus, Nu = nucleolus, Pt = protuberances, rER = rough endoplasmic reticulum, Sec = secretion, Sec1–3 = secretions with increasing electron-densities, Sp = spermatozoon, SSh = secretion sheath, Tr = trachea.

RESULTS

The male genital system of *H. psammophilus* corresponds morphologically to the male genital systems of other solifuge speeies. Two pairs of long testes (four in total) are present. Each pair of testes merges into one vas deferens, whereas in turn the vasa deferentia enter the common genital chamber.

The testes are surrounded by small tracheae and muscle cells (Fig. 1). The muscles are embedded in the layered basal lamina (Fig. 2). The epithelial cells in turn are attached to the basal lamina via hemidesmosomes (Fig. 2). The epithelium of the testis consists of two different parts, namely the glandular and the germinal parts. Cells forming the glandular part are characterized by an extensive amount of rough endoplasmic reticulum. Centrioles arranged in a tandem position can be seen in the apical part of this cell type (Fig. 3). The nuclei are oval or roundish in shape. Apically, the cells are provided with a microvilli border (Fig. 3). Different kinds of secretions are produced by the secretory cells in the glandular part (Figs. 4, 5). Medium electron-dense secretion fills the testis lumen homogenously (see Figs. 3, 7). Additionally, very electron-dense secretions are released as droplets into the lumen (Fig. 5). The sperm cells are located in the germinal part of the testis (Fig. 6). They do not aggregate to groups. Once released individually into the lumen of the testis (Fig. 7), they are transported to the vasa deferentia.

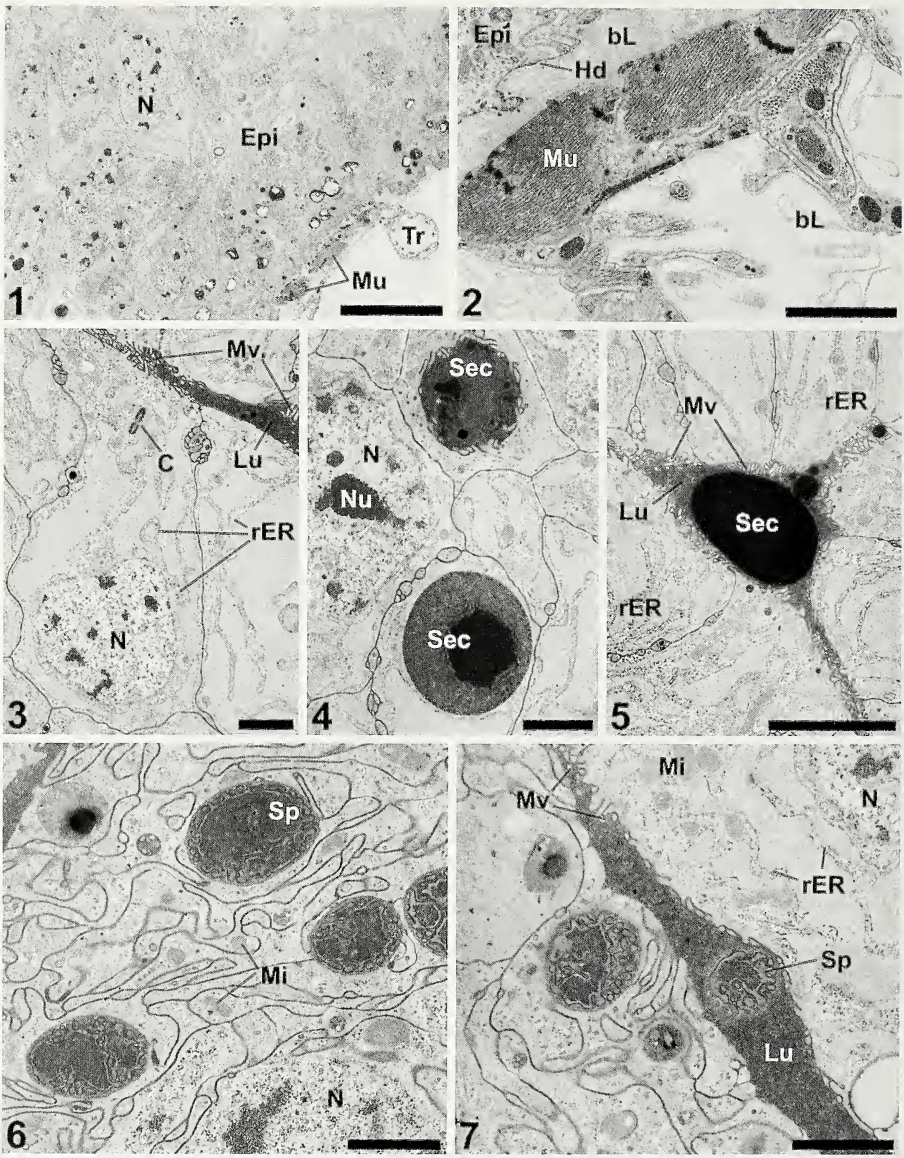
The vasa deferentia are surrounded by an extremely thick muscle layer (Fig. 8). In contrast to the epithelium of the testis, the epithelium of the vasa deferentia is apparently not composed of two parts. The epithelium possesses apically a microvilli border (Fig. 8) and consists of irregularly shaped cells with prominent nuclei. The cell borders strongly interdigitate (Figs. 8, 9). Mitochondria and glycogen granules are also present (Fig. 9). Numerous individual sperm cells and secretions of different electron-densities are visible in the wide lumen (Fig. 10). Depending on the orientation of the section plane, the sperm cells appear oval or circular (Fig. 10). Their average diameter is about 2–2.5 µm. Very rarely, few sperm cells are attached to each other and form small stacks (Fig. 11).

Each individual sperm cell is surrounded by a thin secretion sheath (Fig. 12). The sperm cell forms finger-like protuberances around its entire surface (Figs. 11–15). The chromatin body is very irregularly shaped and exhibits a granulate structure (Figs. 11–15). No nuclear envelope could be observed. Multilamellar bodies and areas with conspicuous electron-dense granules, most likely glycogen, are enclosed by the chromatin body (Fig. 12). The acrosomal complex is deeply embedded in the chromatin body. It is relatively small, consisting of the acrosomal vacuole and the acrosomal filament, the latter originating underneath the small, conical acrosomal vacuole (Fig. 13). The acrosomal filament runs through the chromatin body and apparently coils preferentially around the area where the glycogen particles are located. It does not seem to coil around the chromatin body (Figs. 14, 15).

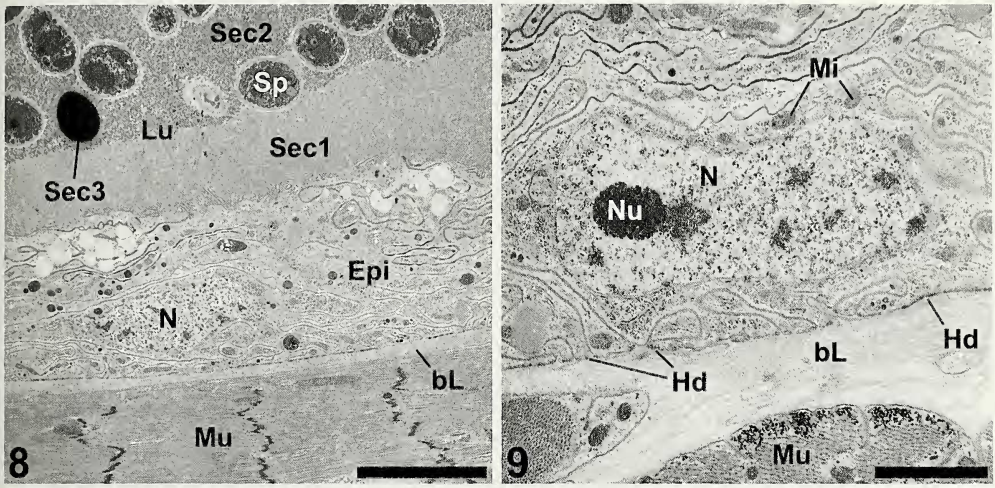
DISCUSSION

It has been shown in former studies that the spermatozoa of solifuges exhibit certain seemingly family-specific morphological characters (Klann et al. 2009), but some similarities were also found between families. The finger-like protuberances and the putative presence of glycogen found here in the sperm cells of *H. psammophilus* are similar to those found in the spermatozoa of Ammotrechidae, some members of Daesiidae and Solpugidae. Multilamellar bodies, previously only observed in *Gluvia dorsalis* (Latreille 1817) (Daesiidae) (Klann, unpubl. data), were also found in the hexisopodid species studied here.

Hexisopodid spermatozoa have a unique feature in that every individual sperm cell is surrounded by its own secretion sheath, thus forming a typical cleistospermium. The formation of sperm stacks in the lumen of the vasa deferentia could possibly be caused by occasional adhesion of the secretion sheaths of different sperm cells. This can also be observed in other solifugid species in which single sperm cells are common. Cleistospermia have not been observed in other solifuge species so far. In contrast to cleistospermia, coenospermia



Figures 1–7.—Testis. 1. Overview of the epithelium of the testis surrounded by muscles and tracheae. Scale bar 10 μm. 2. The epithelium of the testis is attached to the basal lamina via hemidesmosomes. Muscles are embedded in the layered basal lamina. Scale bar 2 μm. 3. In the apical part of epithelial cells constituting the glandular part of the testis centrioles are arranged in tandem position. Large cisternae of rough endoplasmic reticulum are visible. The nucleus is roundish. Scale bar 5 μm. 4. Different secretions are produced by epithelial cells of the testis. Scale bar 2 μm. 5. Secretions are released into the lumen of the testis and most likely transported to the vas deferens. Scale bar 2 μm. 6. Individually distributed spermatozoa are located within the germinal part of the testicular epithelium. Scale bar 2 μm. 7. Single sperm cells are released into the lumen of the testis. In this section the testis lumen separates cells of the germinal part (lower left) from cells of the glandular part (upper right). Scale bar 2 μm.



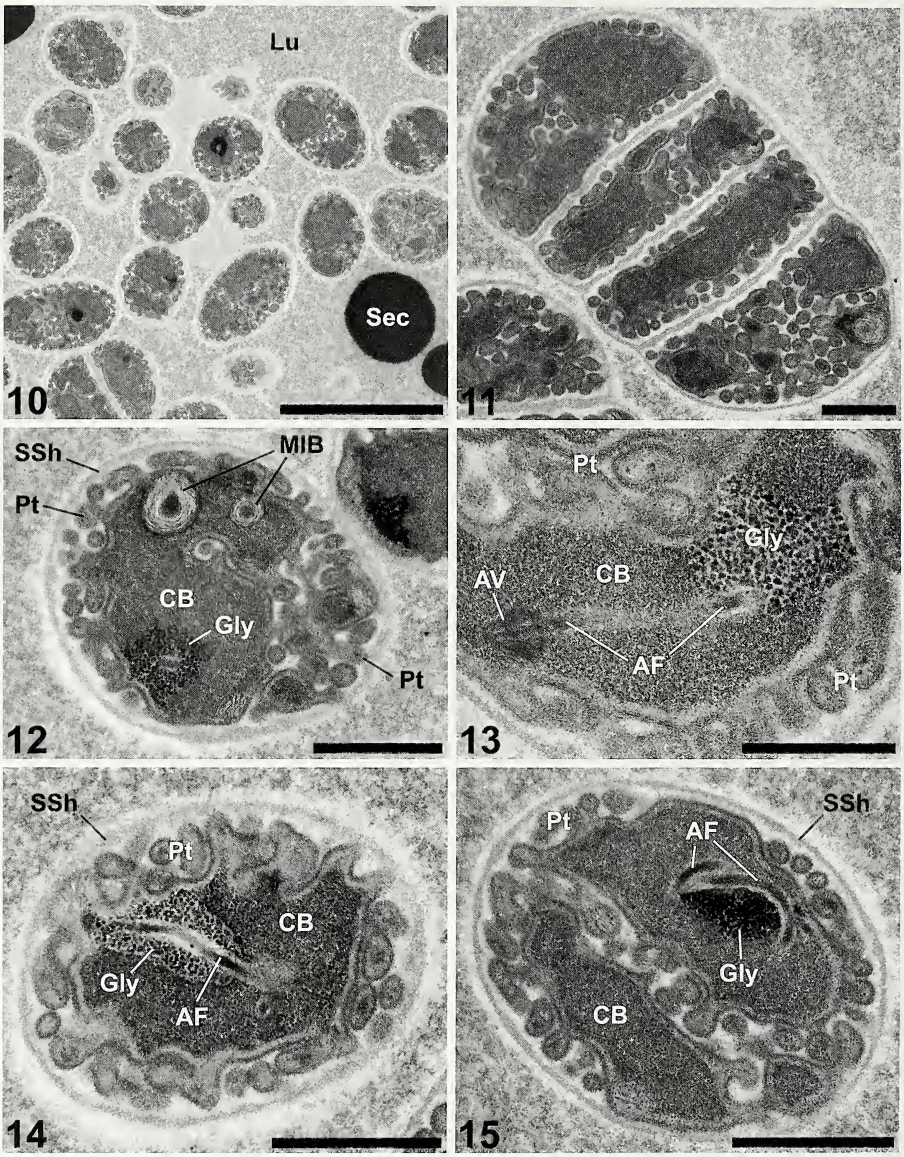
Figures 8, 9.—Vas deferens. 8. The epithelium of the vas deferens is surrounded by a very thick muscle layer. The epithelium itself is comparatively flat. The lumen of the vas deferens is filled with secretions of different electron-densities. Spermatozoa and droplets of high-dense secretion (Sec3) can be found in the homogenous medium-dense secretion (Sec2). Scale bar 5 μm. 9. The epithelial cell is provided with an oval nucleus with a prominent nucleolus. The basal lamina is layered. Note the hemidesmosomes. Scale bar 2 μm.

consist of several sperm cells surrounded by a secretion sheath (Bertkau 1877). This type of transfer form occurs in Galeodidae and certain members of the family Daesiidae, the latter exhibiting the largest spermatozoal variability within the Solifugae (Klann et al. 2009).

In spiders, coenospermia are considered to be ancestral, a hypothesis deriving from the fact that this transfer form occurs in primitive spider taxa, such as Mesothelae and Mygalomorphae (Alberti 1985, 1990; Michalik et al. 2003; Michalik et al. 2004b). Since only fine structural data on spermatozoa from seven solifuge families (including now Hexisopodidae) are known to date (Klann et al. 2009), the evolutionary pathways of this trait in solifuges cannot yet be judged. Nonetheless, our findings suggest that the sperm cells of the investigated hexisopodid most closely resemble the fine structure of sperm cells of the family Solpugidae. This resemblance is not only found in the presence of finger-like membrane protuberanees and glycogen, but also in the organization and form of the acrosomal complex. Both in Solpugidae and in the investigated hexisopodid, the acrosomal vacuole is conical in shape and located within the chromatin body. Interestingly, whereas the current classification places the Hexisopodidae as a separate family within the order, Hewitt (1919) regarded the Hexisopodidae as being a potential subfamily of Solpugidae. It remains to be seen whether the hypothesis of Hexisopodidae as a subfamily of Solpugidae will persist in modern phylogenetic analyses. However, Hewitt's hypothesis, seen in the light of similarities in the fine structure of Solpugidae and Hexisopodidae spermatozoa, could indicate a closer relationship between these two families than is currently recognized. Although morphological similarities do not automatically indicate a close relationship between taxa due to potential convergences, reversals and parallelisms in morphological characters, sperm morphology appear to be very constant, at least within some families of the order Solifugae (Klann et al. 2009). Members of the family Galeodidae, for example, are united by various morphological characters such as shape of the flagellum, opisthosomal "Stigmenkämme" and setose tarsal claws (Roewer 1934). This is also reflected in the morphology of their sperm cells; the sperm cells of different galeodid species are extremely similar and difficult to differentiate from each other. Nonetheless, it is important to note that sperm cells can undergo wide ranges of morphological modifications (Pitnick et al. 2009), and similarities due to, for example, convergences can therefore not be excluded.

In summary, although a set of unique external morphological characters (e.g., fossorial legs) clearly separates the Hexisopodidae from other solifuges, the only autapomorphy found in their sperm morphology was the presence of cleistospermia. Their spermatozoa thus do not reflect their peculiar outer morphology, but rather indicate a closer relationship with the family Solpugidae.

Similar challenges based on spermatozoan ultrastructure have been made in higher level arachnid phylogeny studies. Various authors (Alberti 1980c; Alberti & Peretti 2002; Klann et al. 2005) questioned the commonly accepted clade Haplocnemata (Solifugae + Pseudoscorpiones) based on the coiled flagellate sperm cells of pseudoscorpions versus the aflagellate sperm characteristic of solifuges. These findings have been corroborated by recent molecular studies (Dabert et al. 2010; Pepato et al. 2010) which, independently, recovered the clade Actinotrichida (Acari)+Solifugae, thereby implicating the diphyly of the Acari (Anactinotrichida and Actinotrichida as distinct groups, previously suggested by van der



Figures 10–15.—Spermatozoa. 10. Spermatozoa are distributed individually in the lumen of the vas deferens. Droplets of electron-dense secretions are also visible. Scale bar 5 μm. 11. Sperm cells seldom cling together and form small groups. Scale bar 1 μm. 12. Each individual sperm cell is surrounded by a secretion sheath. Finger-like protuberances apparently surround the entire cell. Multilamellar bodies occur inside the cells. Areas containing glycogen are present within the chromatin body. Scale bar 1 μm. 13. The acrosomal complex is deeply embedded in the chromatin body. The acrosomal vacuole is conical shaped. Scale bar 0.5 μm. 14. In this section the acrosomal filament penetrates the glycogen aggregation. Scale bar 1 μm. 15. This section indicates that the acrosomal filament additionally coils several times around the glycogen aggregation, but it does not coil around the entire chromatin body. Scale bar 1 μm.

Hammen (1989) and Alberti (2005b)) and refuting the Pseudoscorpiones-Solifugae sister group relationship.

It is clear that higher level Solifugae phylogenetic analyses need to be based on quality and carefully considered molecular, as well as a suit of both external and internal morphological characters, if robust phylogenetic hypotheses for this group are to be proposed.

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